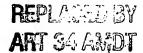


## What is claimed is:

- 1. A gene, which codes for the following protein (a) or (b):
- (a) a protein consisting of an amino acid sequence of any one of SEQ ID NOS: 2, 4, 6, and 8;
- (b) a protein consisting of an amino acid sequence derived from the amino acid sequence of any one of SEQ ID NOS: 2, 4, 6, and 8 by substitution, deletion or addition of at least one or more amino acids, has resistance to a pyrimidinyl carboxy herbicide, and has acetolactate synthase activity.
- 2. An acetolactate synthase protein, which is coded by the gene of claim 1.
- 3. A recombinant vector, which has the gene of claim 1.
- 4. A transformant, which has the recombinant vector of claim 3.
- 5. A plant, which has the gene of claim 1 and has resistance to a pyrimidinyl carboxy herbicide.
- 6. A method for cultivating the plant of claim 5, which comprises cultivating the plant in the presence of a pyrimidinyl carboxy herbicide.
- 7. A method for selecting a transformant cell having the gene of claim 1, which uses the gene as a selection marker.



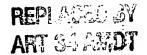
P171H/W548L mutant ALS and P171H/S627I mutant ALS proteins, predicted RS ratios and actual RS ratios were compared. However, it could not be clarified whether resistance stronger than the resistance predicted from the degrees of resistances of each 1-point mutant gene is shown.

Next, inhibition activity by chlorsulfuron (Table 12) revealed the following:

Among the mutant ALS proteins coded by 1-point mutant genes (P171H, R172S, W548L and S627I), W548L mutant ALS protein showed the strongest resistance to chlorsulfuron (RS ratio: 520). P171H mutant ALS protein showed relatively strong resistance (RS ratio: 85), but the degree of resistance of S627I mutant ALS protein was low (RS ratio: 2.4). R172S mutant ALS protein showed resistance only equivalent to that of the wild type ALS protein (RS ratio: 0.85). These results revealed that P171H mutation and W548L mutation in ALS protein are mutations effective in enhancing resistance to chlorsulfuron. Further, R172S mutation in ALS protein was shown to be a silent mutation.

Among the mutant ALS proteins coded by 2-point mutant genes, P171H/W548L mutant ALS protein imparted the strongest resistance (16% inhibition in 100 µM, and RS ratio: >7700), followed by P171H/S627I mutant ALS protein (RS ratio: 760). Unlike the data of inhibition activity by bispyribac-sodium shown in Table 9, in the case of chlorsulfuron, P171H/R172S mutant ALS protein showed a degree of resistance (RS ratio: 420) higher than that of P171H mutant ALS protein. Thus, it was clarified that R172S mutation, which is a silent mutation by itself, enhances the degree of resistance of P171H mutant ALS protein. Further, P171H/W548L/S627I mutant ALS protein also imparted strong resistance (30% inhibition in 500 µM, and RS ratio: >3800).

For P171H/R172S mutant ALS and P171H/S627I mutant ALS proteins, predicted RS ratios and actual RS ratios were compared. For both proteins, the actual RS ratios were significantly higher than the predicted RS ratios. These results revealed that P171H/R172S mutant ALS protein and P171H/S627I



mutant ALS protein showed resistance to chlorsulfuron stronger than that predicted from the degrees of resistances of each 1-point mutant gene.

Next, data of inhibition activity by Imazaquin (Table 13) revealed the following:

Among the mutant ALS proteins coded by 1-point mutant genes (P171H, R172S, W548L and S627I), W548L mutant ALS protein showed the strongest resistance to imazaquin (14% in 100 μM, and RS ratio: >45). S627I mutant ALS protein also showed resistance (RS ratio: 41), but P171H mutant ALS protein showed almost no resistance (RS ratio: 1.5). R172S mutant ALS protein showed resistance only equivalent to that of the wild type ALS protein (RS ratio: 0.98). These results revealed that W548L mutation and S627I mutation in ALS protein are mutations effective in enhancing resistance to imazaquin. Further, P171H mutation and R172S mutation in ALS protein were shown to be silent mutations against imazaquin.

Among the 2-point mutant genes, P171H/W548L mutant ALS protein imparted the strongest resistance (13% inhibition in 100  $\mu$ M, and RS ratio: >45), followed by P171H/S627I mutant ALS protein (RS ratio: 32). The degree of resistance of P171H/R172S mutant ALS protein was almost the same as that of p171H 1-point mutant gene. Further, P171H/W548L/S627I mutant ALS protein also imparted strong resistance (15% inhibition in 100  $\mu$ M, and RS ratio: >45).

For these 2-point ALS mutant proteins and 3-point ALS mutant protein, predicted RS ratios and actual RS ratios were compared. The RS ratio of P171H/S627I mutant ALS protein was significantly higher than the predicted RS ratio (the ratio of the actual RS ratio to the predicted RS ratio was clearly larger than 1). These results revealed that P171H/S627I mutant ALS protein showed resistance to imazaquin stronger than that predicted from the degrees of resistances of each 1-point mutant gene.

Industrial Applicability



The following is an English translation of AMENDMENT, which is submitted to JPO in June 18, 2003, in response to written opinion made out by international preliminary examining authority.

## **AMENDMENT**

To: Commissioner of the Patent Office, Shinichiro OHTA (Examiner, Miyoko SUZUKI)

2. Identification of the International Application

PCT/JP03/01917

2. Applicant

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4. Object of amendment

Claims

- 5. Contents of Amendment
- (1) "A pyrimidinyl carboxy herbicide" of claim 1 is amended as "a bispyribac sodium herbicide, a pyrithiobac sodium herbicide, and a pyriminobac herbicide."
- (2) "A pyrimidinyl carboxy herbicide" of claim 5 is amended as "a bispyribac sodium herbicide, a pyrithiobac sodium herbicide, and a pyriminobac herbicide."
- (3) "In the presence of a pyrimidinyl carboxy herbicide" of claim 6 is amended as "in the presence of at least one or more herbicides selected from the group consisting of a bispyribac sodium herbicide, a pyrithiobac sodium herbicide, and a pyriminobac herbicide."
- (4) Claim 8 is added to Claims.
- 6. List of attached documents
- (1) Page 54, Claims (Pages 67 and 68, Claims, in the English translation)



## DT09 Rec'd PCT/PT0 2.8 SEP 2004

The following is an English translation of AMENDMENT, which is submitted to JPO in July 23, 2003, in response to written opinion made out by international preliminary examining authority.

## **AMENDMENT**

To: Commissioner of the Patent Office, Yasuo IMAI (Examiner, Miyoko SUZUKI)

1. Identification of the International Application

PCT/JP03/01917

2. Applicant

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4. Object of amendment

Specification

- 5. Contents of Amendment
- (1) "RS ratio: 520" on line 25 on page 51 of the JP specification (line 9 on page 64 in the English translation) is amended as "RS ratio: 760."
- (2) "RS ratio: > 3800" on line 11 on page 52 (line 25 on page 64 in the English translation) is amended as "RS ratio: > 38000."
- (3) "14% in 100  $\mu M$  " on line 20 on page 52 (line 7 on page 65 in the English translation) is amended as "16% in 100  $\mu M$  ."
- (4) "RS ratio: 41" on line 21 on page 52 (line 8 on page 65 in the English translation) is amended as "RS ratio: 6.8."
- (5) "RS ratio: 0.98" on line 23 on page 52 (line 10 on page 65 in the English translation) is amended as "RS ratio: 1.0."
- 6. List of attached documents
- (1) Pages 51 and 52 of the specification (Pages 64 and 65 in the English translation)